Rate kinetics of bread bolus disintegration during *in vitro* digestion

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ABSTRACT

The rate of bolus disintegration in the stomach is important in determining gastric emptying, which is correlated with post-food consumption blood glucose levels and satiety. This study examined the effect of various factors on bread bolus disintegration rate, including flour type, gastric juice components, saliva level, and presence of α-amylase in saliva. The kinetics of bolus disintegration were determined by measuring bolus mass retention and fitting the disintegration profiles to a linear-exponential model. Artificially masticated bread was mixed with simulated saliva to form a bolus, which was soaked in simulated gastric juice up to 120 min. The mass retention kinetics during static and agitated soaking followed three distinct profiles depending on bread type. Rye and sourdough bread followed a delayed exponential profile, retaining 91% and 65% of their original mass after 120 min. White bread followed a sigmoidal profile, retaining 33% of its original mass after 120 min. Wheat, almond-wheat, and barley bread followed exponential decay profiles, retaining 11-22% of their original mass after 120 min. Bread type influenced the disintegration profile due to varying levels of water absorption caused by bread structure. The hydrodynamic forces produced by agitating the gastric juice surrounding the bolus or the addition of acid did not produce a significant effect on bolus disintegration for all bread types compared to disintegration in water. Increasing the bolus saliva level increased the moisture content and decreased the cohesive forces in the bolus, increasing the disintegration rate during static soaking.

Keywords: bolus; disintegration; kinetic modelling; bread; in vitro digestion

INTRODUCTION

Digestion begins in the mouth, where ingested food is reduced in size while being lubricated with saliva. The saliva excreted in the oral cavity performs many functions, one of which is assisting chewed food particles adhere to each other as they form a bolus. A bolus will be swallowed after reaching a certain lubrication and particle size threshold [1]. The bolus particle size distribution is dependent on the food being chewed, with median particle sizes ranging from 0.8 – 2.29 mm [2]. When the bolus enters the stomach, peristaltic waves begin, starting a continuous movement of the stomach walls, causing the ingested food particles to collide against each other while they are mixed with gastric secretions [3].

Solid breakdown during gastric digestion is a complex process, comprising of multiple mechanisms. Enzymatic degradation will occur via migration of gastric juice into the food matrix. Physical breakage will be initiated by the mechanical movement of the stomach walls, which will cause food particles to collide with both the stomach walls and with each other.

Previous studies have examined the breakdown of individual food particles during *in vitro* digestion [4]. However, food is actually swallowed in the form of a bolus, which must first be broken apart before the individual particle can be digested. Few studies have concentrated on bolus disintegration during gastric digestion. It has been shown by echoplanar imaging that gastric juice will penetrate into a bolus of viscous locust bean gum while small pieces are fragmented off of the surface [5], but this breakdown process has not been quantified. Knowledge of food bolus disintegration can be used to create a model of bolus breakdown that can be coupled with the existing data about individual particle disintegration, providing a more complete understanding of food breakdown during gastric digestion.

The goal of this project was to determine the driving factors in the disintegration of bread boluses in a simulated gastric environment. Bread made with different types of flour was used as a model food, as it forms a cohesive bolus. The specific objectives were to determine the effect of flour type on the disintegration rate of bread boluses; establish the primary factors responsible for bolus disintegration during gastric digestion by examining the disintegration caused by gastric juice components (acid and enzymes) and

hydrodynamic forces; and study the bolus cohesive forces in the presence of varying amounts of saliva and α-amylase to determine how these changes affect bolus disintegration.

MATERIALS & METHODS

Simulated saliva was prepared with 1 g/L mucin, 2 g/L α-amylase, 0.117 g/L NaCl, 0.149 g/L KCl, 2.1 g/L $NaHCO_3$ and deionized water at pH = 7. Gastric juice was prepared with 1 g/L of pepsin, 1.5 g/L gastric mucin, 8.775 g/L NaCl in deionized water, adjusted to a pH of 1.8 – 2.0 with 0.1 N HCl [4].

Bread was made with 400 mL water, 41 g sugar, 10 g nonfat dry milk powder, 10 g salt, 7.3 g yeast, 30 g butter, and either 596 g white wheat flour (white bread), 653 g wheat flour (wheat bread), 526 g rye flour (rye bread), 501 g barley flour (barley bread), or 326.5 g wheat flour and 326.5 g almond meal (almond-wheat bread). Bread was prepared in a Zojirushi bread maker (model BBCC-X20, Zojirushi American Corporation, Gardena, CA). The bread making cycle used was as follows: 20 min heating at 28°C, 15 min kneading, 45 min rise at 28°C, steam released, 22 min rise at 28°C, dough formed into a ball, 32 min rise at 38°C, 65 min bake at 125°C. After baking, the bread was immediately removed and cooled for one hour. The bread was sliced, the crust removed, and was stored in plastic bags to prevent moisture loss. Bread samples were used for analysis within 48 hours of preparation.

Bread moisture content was measured using AACC Method 44-15A. Bread firmness was measured using AACC Method 74-09 with a 30 mm plunger on 5 slices of bread of each bread type.

Bread was cut into cubes and passed through a meat grinder (KitchenAid USA, St. Joseph, MI) [6] to produce "masticated" bread. Seven g samples of masticated bread were mixed with 2.8 mL simulated saliva for 30 seconds with a mortar and pestle to form each bolus sample. Bolus moisture content was determined in triplicate by drying the samples at 105°C until constant weight. Bolus initial texture was measured using a TA.XT2 Texture Analyzer (Texture Technologies, Scarsdale, NY) on 5 boluses of each treatment. A needle probe (2 mm diameter) was used to penetrate the samples with a displacement speed of 60 mm/min to 10 mm (approximately one third of sample height). Cohesive force was quantified as the area under the curve [7-9].

To determine the breakdown of boluses in a simulated gastric environment, gastric juice was used as the soaking fluid in both static and agitated conditions (120 rpm). Water, gastric juice without acid (pH 3.5), and gastric juice without pepsin were used under static conditions only, resulting in 5 test conditions. For each condition, 6 replicates (boluses) were placed into mesh metal baskets (1-2 mm diameter openings). The bolus samples were placed into a 250 mL beaker filled with preheated fluid at 37°C. They were held at 37°C in a shaking bath (model YB-531, American Scientific Products, McGraw Park, IL), with samples taken every 10 min for one hour, and every 15 min for a second hour. For each sample, the bolus was removed and weighed. Mass retention ratio was calculated as the normalized weight at each time point [4, 10],

$$y_t = \frac{W_t}{W_0}$$

where y_t = mass retention ratio, W_t = weight at time t in grams, and W_0 = initial (directly after preparation) sample weight in grams. The mass retention curves were fit to a linear-exponential model,

$$y(t) = (1 + k \cdot \beta \cdot t) \cdot e^{-\beta \cdot t}$$

where y(t) is the mass retention of the food product at time t, k is a dimensionless constant representing the lag phase, and β (1/min) is a constant representing the curve concavity. This equation has been used to model the disintegration of various food products [10] and changes in stomach volume [11]. The model was fit to the mass retention profiles using Table Curve 2D v4.06 (Systat Software, Inc. San Jose, CA) with a least squares curve fitting algorithm to minimize the sum of squared residuals.

RESULTS & DISCUSSION

Bread Type and Static and Agitated Soaking Effect Bolus Mass Retention

The disintegration profiles for each bread type and soaking condition were fit to a linear-exponential model. Three categories of disintegration profiles were observed: exponential (almond-wheat and barley bread), sigmoidal (white bread), and delayed sigmoidal (rye and sourdough bread) (Figure 1).

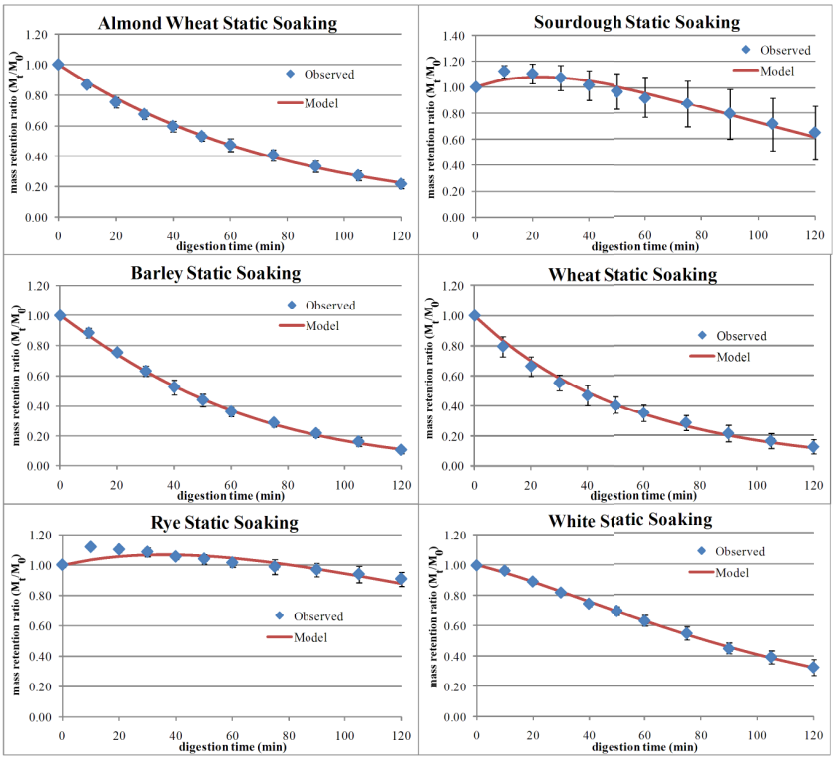

Figure 1. Mass retention profiles from static shaking with observed and modelled values (n = 6). Error bars are ± standard deviation.

The statistical analysis of the linear exponential model parameters (Table 1) indicated that bread type produced a significant effect ($p = 0.001$), while the soaking condition and the soaking condition-bread type interaction were not significant ($p = 0.2178$ and $p = 0.3464$, respectively) in determining the k value. The β values were significantly affected by the bread type ($p = 0.0045$) and the soaking condition-bread type interaction ($p = 0.0424$), but not by the soaking condition alone ($p = 0.2571$). The k value is an indicator of the lag time in the disintegration profile; a larger k value indicates a longer lag time. The k values correspond with the observed disintegration profiles, as the breads exhibiting a delayed-sigmoidal disintegration profile (rye and sourdough bread) have the highest k values. The β values are an indication of the curve concavity and can be thought to represent the rate of decay, with a higher β value signifying a faster rate of decay.

Table 1. Linear-exponential model parameters for static and agitated soaking in gastric juice.

		k			β		
Almond-Wheat	Static	0.000	±	0.001	0.001	±	0.012
	Agitated	0.184	±	0.411	0.025	±	0.048
Barley	Static	0.452	±	0.225	0.004	±	0.025
	Agitated	0.455	±	0.379	0.020	±	0.046
Rye	Static	1.477	±	0.096	0.001	±	0.009
	Agitated	1.030	±	0.080	0.001	±	0.013
Sourdough	Static	1.510	±	0.335	0.003	±	0.015
	Agitated	0.867	±	0.427	0.002	±	0.012
Wheat	Static	0.004	±	0.003	0.002	±	0.018
	Agitated	0.004	±	0.005	0.001	±	0.033
White	Static	0.730	±	0.048	0.002	±	0.017
	Agitated	0.517	±	0.137	0.005	±	0.019

Moisture absorption by a solid material will cause changes in the material, as the internal structure is altered. In coated tablets, water uptake causes swelling and increases the tablet internal forces until the tablet begins to disintegrate [12]. Water uptake also causes accelerated erosion and disintegration [13]. Absorption

of gastric juice by the bolus may also have an effect on bolus breakdown; the amount of gastric juice absorbed by the bolus during soaking may depend on the initial bolus moisture content and physical structure. The moisture content and firmness of the bread samples will determine the bolus properties. Bread firmness varied from 583 – 3772 g, with white and sourdough bread having the lowest firmness values, and almond-wheat, barley, and wheat having the highest firmness values (Table 1).

Table 2. Firmness and wet basis moisture content. Values are listed as the average (n = 5) ± standard deviation.

Bread Type	Firmness (g)			Wet Basis Moisture Content		
Almond Wheat	3772	±	692	35.4%	±	0.2%
Barley	1972	±	235	29.2%	±	6.9%
Rye	1848	±	184	37.6%	±	0.3%
Sourdough	599	±	85	37.7%	±	1.0%
Wheat	1979	±	427	30.9%	±	1.6%
White	583	±	208	35.9%	±	0.2%

The differences in disintegration rate between bread types can be explained by examining the firmness and moisture content of the different breads. The faster disintegrating breads (wheat, almond-wheat, and barley) had lower initial moisture contents compared to the slower disintegrating breads (rye, sourdough, and white), so they were more inclined to absorb water. These breads also had high firmness values, indicating that their structure was more compact. As the fastest disintegrating breads absorbed gastric juice, their firm structure was quickly weakened, surmounting the critical stresses in the bread matrix, causing the breads to break apart and dissolve at a rapid rate. The dissolution could have been accelerated by the large amount of gastric juice penetrating into the bolus, exposing more bolus surface area to the enzymatic and acidic conditions of the gastric juice. The converse is also true for the slower disintegrating breads. This suggests that both the moisture content and bread structure, as quantified by firmness, can explain the differences seen in bolus disintegration rate.

Disintegration Rate Comparison with Glycemic Index Values

The relative disintegration of the bread boluses can be compared with observed glycemic index (GI) values for each bread type to determine if the *in vitro* disintegration follows the same pattern as observed *in vivo*. The relative order of glycemic index, from lowest to highest is: white bread + almonds < sourdough < rye < barley < wheat and white bread. The relative order of disintegration, from slowest to fastest is: rye < sourdough < white < barley < wheat and almond-wheat bread. The results from this study agree with the published GI values in the observation that rye and sourdough bread have the slowest disintegration (and hence a lower GI value), and also that wheat bread has a fast disintegration [14].

One contradiction between these results is that almonds and white bread resulted in a low GI value, while almond-wheat bread had one of the fastest disintegration rates. This disparity could be caused by the difference in almond structure, as almond meal (used to make the almond-wheat bread) is already a finely ground flour, while whole almonds still require a great deal of structural breakdown before they will be fully digested [15]. White bread also shows a difference in relative digestion compared to the published GI values. According to the GI values, white bread should have one of the fastest disintegration rates, but white bread showed an intermediate disintegration rate. The white bread in this study was made with only white wheat flour, whereas in conventional white flour there are other ingredients added to improve the baking quality of the flour which could have caused the differences in disintegration rate. Bread bolus disintegration shows a good agreement with the relative differences in glycemic index, suggesting that the GI value of bread products is heavily dependent on the bread bolus disintegration.

Gastric Juice Variation effect on Bolus Mass Retention

Static soaking in gastric juice caused significant disintegration for all of the bread types tested, although it is not clear which component caused this disintegration. To determine which factor is responsible for the bolus disintegration, boluses were soaked in water, gastric juice without added acid, and gastric juice without added pepsin. The mass retention profiles for all of the disintegration tests were fit to the linear-exponential model previously described. The soaking condition and the method (no acid, regular gastric juice, and water) were not significantly different, but the no acid and static conditions were different from the no pepsin soaking condition ($p = 0.0328$ and $p = 0.0428$, respectively). Since the k value from the linear exponential fit is a measure of the lag time before the disintegration occurs, the greatest effect on the lag time

is from the gastric juice without pepsin, suggesting that pepsin is the most important ingredient in the gastric juice composition that affects the disintegration of bread boluses during static soaking. The β values were not the significantly different for the different soaking conditions. This shows that although the soaking condition has a significant effect on the lag phase (k), it does not have an effect on the rate of disintegration and the curve concavity (β).

Variations in Saliva

The rate of saliva excretion in the oral cavity is a result of many factors, such as circadian rhythm [16], taste factors, (sweet, salty, and bitter) [17], and food material properties [18]. During periods when none of these stimuli are present, saliva is still secreted; however, the amount of secretions can be quite variable. The variation in unstimulated saliva flow rate in a group of similar aged healthy subjects can range from 27-44% over a six hour period [19]. This large inherent variation in saliva will lead to varying amounts of saliva incorporated into food boluses upon formation. Particularly in starchy foods, the amount of saliva may play a major role on the digestion of the food due to its high level of α-amylase.

To determine the effect of saliva amount and presence of α-amylase on bread bolus disintegration, boluses of saliva levels varying from 0.2-1.0 mL/g bread were tested, as well as boluses with 0.4 mL/g saliva with and without α-amylase. The cohesive force in the boluses varied with bread type, amount of saliva, and presence of α-amylase (Figure 2). The cohesive force at 1.0 mL/g saliva was assumed to be approximately 0 N·s, as the boluses produced with this amount of saliva did not produce any resistance during testing and are not shown on the figure. The mechanism by which the saliva amount alters the bolus texture can be attributed to two causes: the cohesive force generated by lubrication of bread particles with the addition of saliva, and

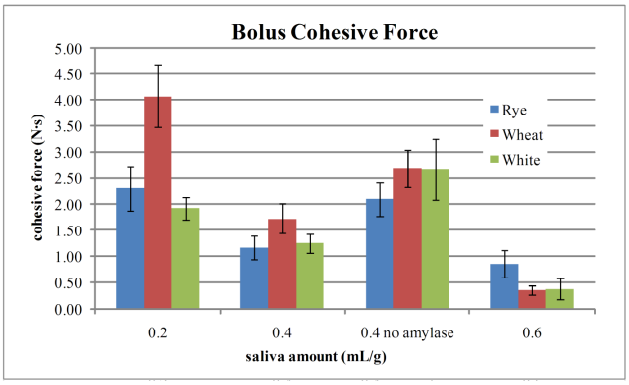

Figure 2. Bolus cohesive force for varying saliva levels (n = 5).

disintegration due to α-amylase. Prinz and Lucas [20] hypothesized that a bolus will form and stick together as saliva is mixed with masticated pieces of food. The cohesive force will vary depending on the number of chews, which will initially dictate the particle size, and continuously influence the amount of saliva in the bolus. After the optimal level of saliva is reached, the bolus is given more saliva than it can absorb, causing a decrease in cohesive force. Starch digestion by α-amylase could also cause a decrease in cohesive force. The effect of α-amylase can be seen by comparing the cohesive force levels for the boluses made with equal amounts of saliva (0.4 mL/g) with and without α-amylase (Figure 2). In the short period of time (<5 min) from formation to texture analysis, the α-amylase in the saliva already caused a significant decrease in the bolus cohesive force. Since α-amylase is inactivated at a low pH, such as those found in the stomach, it had previously been thought that salivary α -amylase did not play a major role in starch digestion; the majority of starch was thought to be digested by pancreatic amylase [21]. However, it has been shown that a bolus may remain undiluted by gastric juice for time periods over an hour in the stomach [5]. The results of the current study show that bolus texture can be influenced by only a few minutes of contact with α -amylase, therefore it is probable that upon entering the stomach, if the bread bolus does not immediately become diluted by gastric juice, the α-amylase will still be digesting the inner part of the bolus.

The mass retention profiles from boluses at varying saliva levels were fit to a linear-exponential model. The k values were statistically significant in terms of bread type ($p < 0.0001$) and saliva level-bread type interaction ($p = 0.0425$). In all three bread types, increasing the amount of saliva caused the k value to decrease. The k value represents the lag phase of the disintegration, meaning that by increasing the bolus saliva level, the initial lag phase was decreased. The magnitude of this decrease varies with bread type. The β

values were statistically significant in terms of bread type ($p = 0.0038$) and saliva level ($p = 0.0111$), but not in terms of saliva level-bread type interaction ($p = 0.1265$). The β value describes the curve concavity, representing the rate of disintegration, suggesting that the increase in saliva increased the rate of bolus disintegration.

CONCLUSION

The disintegration rate and type of disintegration profile (delayed-sigmoidal, sigmoidal, or exponential) of bread boluses in both static and agitated soaking were significantly influenced by bread type. Bolus disintegration is affected by many factors, including the bread firmness and moisture content, saliva level, and soaking conditions.

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